Regulation of Human 5-Aminolevulinate Synthase 2 Expression During Erythroid Differentiation and Its Role in X-Linked Sideroblastic Anaemia

A thesis submitted to the University of Adelaide for the degree of Doctor of Philosophy

by

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March, 2003

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THESIS SUMMARY

Haem is required for many cellular processes including haemoglobin synthesis in erythroid cells, where the majority of the haem in the body is produced and utilised. Its synthesis requires tight regulation to prevent toxic levels of free haem from arising. 5-Aminolevulinate synthase 2 (ALAS2) is the first and rate limiting enzyme in the biosynthesis of haem in erythroid cells. Thus, the regulation of ALAS2 expression is critical for maintaining and controlling haem production during the process of erythroid cell differentiation. A major aim of this study was to identify the regulatory elements within the ALAS2 gene that are involved in controlling transcription of ALAS2 during erythropoietin (Epo) stimulated erythroid differentiation. In order to investigate the regulation of ALAS2 transcription in the context of red blood cell maturation, an erythroid cell line, J2E, that terminally differentiates in response to Epo treatment was employed in this study.

Human ALAS2 promoter deletion studies demonstrated that the first 293 bp of the proximal promoter was sufficient to enhance transcription in response to Epo induced differentiation of the J2E cells. Introns 1 and 8 exhibited Epo responsive enhancer activity with intron 8 proving to be the stronger transcriptional activator in response to Epo. Transcription factor binding sites located in the 3' end of intron 8 that are critical to intron 8 Epo responsive enhancer activity were also identified. Preliminary studies on the effect of the coactivators CREB binding protein (CBP) and p300 on ALAS2 expression in response to Epo stimulation were conducted and suggested a potential involvement of these factors in regulating ALAS2 transcription.

Defective haem synthesis, as a result of point mutations in the human ALAS2 gene, has been implicated in a blood disorder called X-linked sideroblastic anaemia (XLSA). XLSA is characterised by the presence of iron loaded mitochondria surrounding the nucleus in erythroblasts of the bone marrow. Anaemia, associated with a cycle of ineffective erythropoiesis that is linked to increased intestinal iron absorption and the secondary effect of iron overload is exhibited by XLSA patients. Point mutations in the ALAS2 gene of XLSA probands have been identified and two associated mutations, C1215G in exon 8 and C1283T in exon 9 were selected as a basis for a murine model for XLSA. Thus, the aim of this project was to develop an animal model for XLSA to investigate the role of ALAS2 in this blood disorder and the associated defects in iron metabolism. A gene targeting approach using

embryonic stem (ES) cells was employed and several strategies trialed. A potential ALAS2 targeted ES cell line containing the C1159G point mutation in exon 8 (equivalent to the human mutation) was generated, with further characterisation required.